12 *Immune Thrombocytopenic Purpura: Etiopathogenesis, Clinical Features and Diagnosis*

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Abstract: Immune thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by increased platelet destruction. It is seen in children where it is usually acute and self-limited, and also occurs in adults, where it is insidious in onset and usually chronic in nature. Among adults it is thought to predominantly affect young women, although this view has been challenged by recent epidemiological studies. The pathogenesis is complex. There is formation of platelet antibodies with a complex interaction between T cells, B cells and antigen-presenting cells. Platelet destruction occurs predominantly, but not exclusively, in the spleen.

The usual concept of pathogenesis has always invoked the idea of increased compensatory platelet production in the pathogenesis of ITP. Recent studies have suggested that impaired platelet production may also occur in many patients. These new mechanisms have implications for novel therapeutic agents. The clinical manifestations of ITP are due to low platelets, leading to mucocutaneous bleeding. Additional clinical features should lead to search for alternative diagnosis. There is no single diagnostic test for ITP. The diagnosis is based on exclusion of other causes of thrombocytopenia based on history, clinical features, blood counts and peripheral blood smear examination. In the majority of patients further investigations are not required, but intelligent application of specific tests is essential to avoid an erroneous diagnosis.

EPIDEMIOLOGY

Immune thrombocytopenic purpura (ITP) is classified as primary or as secondary to an underlying disorder and as acute, if it is of six months or less in duration, or chronic. Acute ITP is seen mainly in childhood, and often follows a viral illness or immunization. The incidence of childhood ITP is similar to that of acute leukemia, at about 4 per 100,000 children per year.[1] Adult-onset and childhood-onset immune thrombocytopenic purpura are strikingly different. Affected children are young (peak age, approximately 5 years) and previously healthy, and they typically present with the sudden onset of petechiae or purpura a few days or weeks after an infectious illness. Boys and girls are equally affected. In more than 70% of children, the illness resolves within six months, irrespective of whether they receive therapy.

By contrast, immune thrombocytopenic purpura in adults is generally chronic, the onset is often insidious, and approximately twice as many women as men are affected.[2] ITP in adults is considered a disease predominantly of women of childbearing age between 18 to 40 years. Two recent surveys suggest that this perception is not correct. In a survey of ITP from Denmark, using International Classification of Disease (ICD) codes at hospital discharge over a 22-year period, the female-male ratio was 1.7, the median age at diagnosis was 56 years, and the incidence of ITP

increased with age.[3] In another prospective cohort analysis of newly presenting adults with platelet counts less than 50,000/ cu mm in the Northern Health Region of the United Kingdom, the female-male ratio was 1.2 and the age-specific incidence was highest among those older than 60 years.[4]

PATHOGENESIS

ITP is a heterogeneous disease characterized by increased platelet destruction and thrombocytopenia. A number of features suggest this destruction is immune-mediated and that it may involve not only the destruction of the platelet, but also inhibition of platelet release by the megakaryocyte. The exact mechanism of the immune dysfunction, however, is generally not known.

Questions Relating to ITP which Require Further Research

a. What initiates ITP?
b. What is the underlying defect resulting in the accelerated platelet destruction?
c. What maintains the disease?
d. Why do some people recover while others have persistent thrombocytopenia?
e. What is the mechanism of the thrombocytopenia in different patients?
f. Why does ITP manifest with varying severity of bleeding with similar platelet counts in different patients?
g. Why does response to different therapies vary in different patients?
h. Does ITP associated with other diseases, such as systemic lupus erythematosus (SLE) and hypothyroidism have the same pathology as primary (idiopathic) ITP?

ANTIPLATELET FACTOR IN PLASMA

About 50 years ago, Harrington first demonstrated that a factor in plasma from patients with ITP induced thrombocytopenia in normal subjects.[5] This was later identified as antiplatelet autoantibody against glycoproteins in the platelet membrane. It was shown that the thrombocytopenic factor was in the immunoglobulin (Ig) G-rich serum fraction, and was conjectured to be an antiplatelet antibody.[6] Platelet-associated IgG was first quantified by Dixon and Ross, who demonstrated a high degree of sensitivity, more than 85-90%, in patients with ITP but with a low specificity.[7]

The specificity of the antiplatelet antibodies to individual platelet glycoproteins (GP), such as GPIIb/IIIa, was demonstrated using the platelet immunofluorescent test (PIFT). [8] Current studies using platelet GP-specific assays, modeled on the monoclonal antibody immobilized platelet antigen (MAIPA), maintain a high specificity, 85-90%, at the expense of a lower sensitivity, 50-70%.

These antibodies rarely interfere with the function of platelets, as patients with ITP very infrequently suffer from major bleeding when their counts are more than 500,000/cu mm. The destruction of platelets by the mononuclear phagocyte system (MPS) seems to result in the presentation of additional platelet antigens to the immune system by the antigen-presenting cells with epitope spreading. Hence, patients with chronic ITP often have antiplatelet antibody with specificity directed to multiple GP; i.e. anti-GP IIb/IIIa and anti-GPIb/IX and anti-GPIa/IIa.[2]

INITIAL DEVELOPMENT OF ANTIPLATELET ANTIBODIES

The initiation of antiplatelet antibodies, however, is not clear. The V_H3-30 heavy chain has been found to be highly represented among platelet-reactive Fab fragments from patients with ITP when compared with its prevalence in the general library.[9] This V_H3-30 heavy chain gene has also

been implicated in the pathogenesis of diseases such as autoimmune hemolytic anemia (AIHA), SLE, chronic lymphocytic leukemia (CLL), common variable immunodeficiency (CVID) and human immunodeficiency virus (HIV) infection. Some studies have shown light chain restriction of antibodies in patients with ITP. These suggest that antiplatelet autoantibodies may be clonally restricted and that antiplatelet antibodies are produced from a limited number of B-cell clones. As antigen-driven affinity selection and somatic mutation are involved, this indicates T cell-driven antibody production.[6]

T Cell Abnormalities

In ITP, a number of T cell abnormalities have been demonstrated. There are three likely mechanisms involved.

a. A number of studies suggest a Th1 bias, compared with Th2, in adults with chronic ITP. An increased numbers of HLA-DR + T cells, increased soluble IL-2 receptors, and a cytokine profile suggesting the activation of precursor helper T and type 1 helper T cells have been described.[10]
b. T cells may be involved in the release of cytokines that interfere with megakaryocyte maturation and/or platelet release.
c. There is evidence to suggest a direct cytotoxic effect of T cells. DNA microarray screening has revealed increased expression of several cytotoxic genes, such as granzyme A, granzyme B and perforin, as well as increased expression of genes involved in the Th1 cell response, such as INFgamma and IL-2 receptor-beta in a small number of patients with ITP when compared with controls by Olsson, et al.[11]

Platelet Production

It has always been assumed that there is compensatory but inadequate increased platelet production in patients with ITP. Two initial studies of platelet survival suggested that platelet production was increased but only by one to three-fold and not in all patients. In the early 1980s, the survival time of circulating autologous platelets in several studies using 111-indium showed that platelet survival was longer than expected (near normal). Therefore, platelet turnover and, by inference, platelet production was found either to be decreased or at best normal in approximately two-thirds of patients with ITP.[6]

Recent studies with a thrombopoietic agent in ITP have suggested that there is a dose-dependent increase in the platelet count following a single or multiple injections. Therefore, it appears that antiplatelet glycoprotein antibodies and, possibly, antiplatelet T cells have effects on megakaryocytes as well as on platelets, probably contributing to thrombocytopenia in a substantial number of patients.

Fc Receptors and Role of Spleen

Platelets coated with IgG autoantibodies undergo accelerated clearance through Fc gamma receptors that are expressed by tissue macrophages, predominantly in the spleen and liver.[2]

Virus-associated ITP

Acute ITP often occurs following a viral illness. The viral infection is cleared normally but initiates ITP, probably via molecular mimicry or B-cell stimulation. ITP has been associated with infection with HIV, hepatitis C virus (HCV) and EBV infection.

Bacteria-associated ITP: *H. pylori*

A number of studies have suggested an association between *H. pylori* and ITP. Studies from Italy and Japan have suggested an increased prevalence of *H. pylori* in patients with ITP and shown a

response rate of between 38% and 73% in patients in whom *H. pylori* is eradicated. However, studies from France, Spain and the USA have not replicated these results, suggesting that *H. pylori* may have different pathogenicity depending on the area studied.[6] Patients with newly diagnosed ITP and those with milder thrombocytopenia may be more likely to increase their platelet counts following eradication of *H. pylori*.[12]

Genetics

Immune thrombocytopenic purpura has been diagnosed in monozygotic twins and in several families, and a propensity for autoantibody production in family members has been noted. However, many studies have failed to demonstrate a consistent association between immune thrombocytopenic purpura and specific major-histocompatibility-complex class I or class II polymorphisms.[2]

Clinical Features

Childhood ITP

In childhood, ITP typically develops in a healthy child with a preceding history of viral infection, with petechiae or mucosal bleeding, is self-limited with spontaneous recovery in the majority.

Adult ITP

The illness typically has an insidious onset, with no preceding viral or other illness. Symptoms and signs are highly variable and range from the fairly common asymptomatic patient with mild bruising, mucosal bleeding (e.g. oral or gastrointestinal tract) to frank hemorrhage from any site, the most serious of which is intracranial. Overall, bleeding symptoms are uncommon unless the ITP is severe, with platelet counts < 30,000/cu mm). ITP *per se*, does not cause anemia. If there is chronic blood loss, features of iron deficiency anemia would also develop. As nutritional anemia is common in our population, any significant chronic bleeding is likely to aggravate iron deficiency with fall in hemoglobin.

Major severe bleeding occurs mainly in patients with platelet counts < 20,000/cu mm, usually < 10,000/cu mm. Younger patients tolerate low platelet levels better than the elderly. Any bleeding is aggravated by use of antiplatelet drugs like aspirin, viral infections or trauma.

Diagnostic Approach for Adults (Table 12.1)

Clinical History

1. Determine the type of bleeding and distinguish 'platelet-type' from 'coagulation-type' hematomas in the muscles or joints.
2. Assess the severity, extent and duration of bleeding.
3. Determine the presence of medical conditions, which may be associated with autoimmune thrombocytopenia, e.g. drugs, human immunodeficiency virus (HIV) infection, other autoimmune disorders, malignancy.
4. Conditions, which may aggravate risk of bleeding: peptic ulcer, renal stones, and severe hypertension.

Physical Examination

1. Assess the type, severity and extent of bleeding.
2. Exclude conditions that might cause non-immune thrombocytopenia. It should be noted that a palpable spleen has been reported to occur in less than 3% of adult patients with ITP and up to 10% children with ITP.

3. Determine the presence of medical conditions that may be associated with autoimmune thrombocytopenia, e.g. HIV infection, other autoimmune disorders, malignancy.

Laboratory Investigations

The diagnosis of ITP is based principally on the exclusion of other causes of thrombocytopenia using the history, physical examination, blood count, peripheral blood film, autoimmune profile and other investigations. The common causes of thrombocytopenia which can be considered in the differential diagnosis of ITP are given in Table 12.2.[1,13,14]

The finding of thrombocytopenia on a routine blood count may be the first indication of ITP. There may be associated iron deficiency anemia if blood loss is substantial, such as seen in patients with menorrhagia, and the erythrocytes may be hypochromic. Thrombocytopenia should be confirmed by examination of the blood film. The blood film should also be examined to exclude non-immune thrombocytopenia such as those associated with acute or chronic leukemia, myelodysplasia, megaloblastic anemia, microangiopathic anemia, inherited thrombocytopenia and pseudo-thrombocytopenia. An autoimmune screen should be carried out to exclude other underlying autoimmune diseases, but in typical cases is unlikely to yield results. If the history, physical examination, blood count and blood film examination are consistent with the diagnosis of ITP with no atypical findings, it can be argued that additional investigations such as bone marrow examination and assays for platelet antibodies are unnecessary. The typical findings on a peripheral blood smear are shown in Table 12.3.[1] The bone marrow classically shows normal or increased megakaryocytes, and excludes other bone marrow abnormalities.

Table 12.3: Peripheral blood smear examination in ITP

Features consistent with diagnosis of ITP
1. Thrombocytopenia: Platelet size normal or larger. Predominant giant platelets (approaching the size of erythrocytes) should be absent.
2. Normal red cell morphology.
3. Normal white blood cell morphology.
Features not consistent with diagnosis of ITP
1. Predominant giant platelets.
2. Red blood cell poikilocytosis, schistocytosis, macrocytes, nucleated red cells, polychromatophilia (unless as a response to bleeding).
3. Leukocytosis or leukopenia, immature or abnormal leucocytes. In childhood ITP atypical lymphocytes or eosinophilia may be seen.

Role of Bone Marrow Examination

The role of bone marrow examination in ITP is a controversial issue. The American Society of Hematology (ASH) published its recommendations for ITP in 1996.[1] The ASH panel reached a consensus that bone marrow should be done for patients older than 60 years or where splenectomy was being considered. It, therefore, often assumed that the ASH panel recommended against routine bone marrow examination in ITP, the ASH recommendations consider a complete blood count and examination of the peripheral smear as essential. In India, many "experts" routinely quote the ASH recommendations as irrefutable. Unfortunately, no work has been done to validate these recommendations in the Indian context. It is an unfortunate fact that in most centers in India, the hematology cell counters are not available, or quality control is not ensured. The peripheral smears are often not seen, or the concerned person does not have the requisite experience.

Many experts do recommend a bone marrow examination in ITP. Cines and Blanchette recommend a bone marrow examination in patients over 40 years of age, in patients with atypical features (e.g. those with additional cytopenias), or in those who do not have a brisk or robust

response to therapy.[2] A marrow examination is mandatory in atypical cases, such as those with lassitude, protracted fever, bone or joint pain, unexplained macrocytosis, or neutropenia.[2]

There is a general consensus that bone marrow examination is not necessary in children if management involves observation or intravenous immune globulin.[15] Many pediatric hematologists recommend that an aspiration be performed before starting corticosteroids to rule out the rare case of acute leukemia.[16] The British guidelines for ITP in children recommend that the bone marrow be examined before steroid therapy is given.[13]

In India, there are no consensus guidelines for ITP management. In our opinion it is advisable to do a bone marrow examination in adults when ITP is suspected, unless all the conditions laid out by the ASH panel are fulfilled. In children who have a typical presentation it can be avoided. In either age groups, if the planned treatment is with intravenous immunoglobulin (IVIG) or anti-D, bone marrow may be avoided, as a response suggests underlying ITP. If corticosteroids are used, they may mask an underlying lymphoblastic leukemia or lymphoproliferative disease, and a bone marrow examination is recommended even in children.[13]

Antiplatelet Antibodies

Platelet associated antibodies are neither sensitive nor specific for ITP and are not recommended in the diagnostic work-up of ITP.[13]

Helicobacter pylori

Despite the limited data suggesting an association with *H pylori* infection and ITP, routine testing for *H pylori* in every case of ITP is not recommended.[14] In patients who are refractory to therapy, or who have gastrointestinal symptoms, it is worthwhile performing serological assays and breath tests aimed at detecting the microorganism.[13,14]

Specialised Investigations

Measurement of the thrombopoietin (TPO) levels may be informative in complex cases of thrombocytopenia, and are particularly useful in distinguishing between reduced production of platelets (high TPO level) and increased destruction of platelets (normal level). However, this assay is not available in routine practice, and is not recommended as part of the routine investigation of ITP.[13]

CONCLUSIONS

Immune thrombocytopenic purpura is an autoimmune bleeding disorder mediated by autoantibodies. Platelets coated with IgG autoantibodies undergo accelerated destruction predominantly in the spleen and liver. A compensatory increase in platelet production occurs in most patients. In others, platelet production appears to be impaired, as a result of either intramedullary destruction of antibody-coated platelets by macrophages or the inhibition of megakaryopoiesis. Thus, improving our understanding of the interaction and relative contribution of humoral and cell-mediated mechanisms is essential for developing antigen-specific immunotherapies for the treatment of this disorder.

REFERENCES

1. George JN, Woolf SH, Raskob GE, et al. Idiopathic thrombocytopenic purpura: a practice guideline developed by explicit methods for the American Society of Hematology. Blood 1996;88:3-40.
2. Cines DB, Blanchette VS. Immune thrombocytopenic purpura. N Engl J Med 2002;346:995-1008.
3. Frederiksen H, Schmidt K. The incidence of idiopathic thrombocytopenic purpura in adults increases with age. Blood 1999;94:909-13.

4. Neylon AJ, Saunders PW, Howard MR, Proctor SJ, Taylor PR. Clinically significant newly presenting autoimmune thrombocytopenic purpura in adults: a prospective study of a population-based cohort of 245 patients. Br J Haematol 2003;122:966-74.
5. Bromberg ME. Immune thrombocytopenic purpura—the changing therapeutic landscape. N Engl J Med 2006;355:1643-5.
6. Cooper N, Bussel J. The pathogenesis of immune thrombocytopaenic purpura. Br J Haematol 2006;133:364-74.
7. Dixon RH, Rosse WF. Platelet antibody in autoimmune thrombocytopenia. Br J Haematol 1975;31:129-34.
8. van Leeuwen EF, van der Ven JT, Engelfriet CP, von dem Borne AE. Specificity of autoantibodies in autoimmune thrombocytopenia. Blood 1982;59:23-6.
9. Roark JH, Bussel JB, Cines DB, Siegel DL. Genetic analysis of autoantibodies in diopathic thrombocytopenic purpura reveals evidence of clonal expansion and somatic mutation. Blood 2002;100: 1388-98.
10. Andersson PO, Wadenvik H. Chronic idiopathic thrombocytopenic purpura (ITP): molecular mechanisms and implications for therapy. Expert Rev Mol Med 2004;6:1-17.
11. Olsson B, Andersson PO, Jernas M, et al. T-cell-mediated cytotoxicity toward platelets in chronic idiopathic thrombocytopenic purpura. Nat Med 2003;9:1123-4.
12. Stasi R, Rossi Z, Stipa E, Amadori S, Newland AC, Provan D. Helicobacter pylori eradication in the management of patients with idiopathic thrombocytopenic purpura. Am J Med 2005;118: 414-9.
13. Guidelines for the investigation and management of idiopathic thrombocytopenic purpura in adults, children and in pregnancy. Br J Haematol 2003;120:574-96.
14. Cines DB, Bussel JB. How I treat idiopathic thrombocytopenic purpura (ITP). Blood 2005;106:2244-51.
15. Calpin C, Dick P, Poon A, Feldman W. Is bone marrow aspiration needed in acute childhood idiopathic thrombocytopenic purpura to rule out leukemia? Arch Pediatr Adolesc Med 1998;152: 345-7.
16. Vesely S, Buchanan GR, Cohen A, Raskob G, George J. Self-reported diagnostic and management strategies in childhood idiopathic thrombocytopenic purpura: results of a survey of practicing pediatric hematology/oncology specialists. J Pediatr Hematol Oncol 2000;22:55-61.